



Routine DNA extraction from fish tissues and subsequent PCR for DNA barcoding is time consuming and laborious, limiting the potential for rapid, high-throughput analysis.

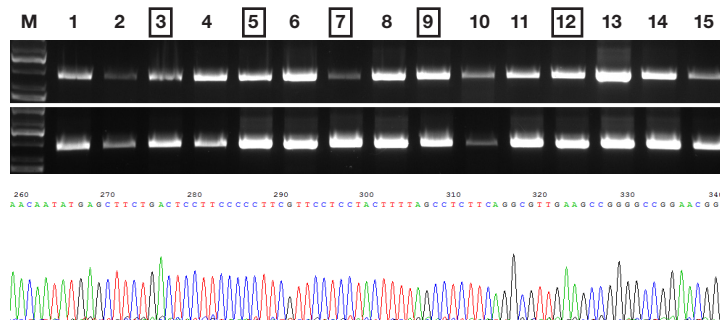
KAPA Express Extract, combined with KAPA2G Robust HotStart ReadyMix, is ideally suited for the routine extraction and amplification of DNA extracted from fish tissues. The novel KAPA Express Extract enzyme and buffer offer an easy and fast way to prepare PCR-ready DNA. KAPA2G Robust HotStart ReadyMix contains an engineered DNA polymerase tolerant to common PCR inhibitors, offering reliable amplification of DNA extracted from fish tissues.

## Introduction

"DNA barcoding" is rapidly gaining support as a quick, cost-effective and broadly applicable tool for species identification. The DNA barcoding process targets a standardized fragment of the cytochrome c oxidase I (COI) mitochondrial gene, which is amplified and sequenced to yield a reference sequence or "barcode". This strategy has found particular application in global efforts to gather DNA barcodes for fish species listed in the FISH-BIO and Marine Code of Life databases (see [www.fishbol.org](http://www.fishbol.org) and [www.marinebarcoding.org](http://www.marinebarcoding.org), respectively). These projects have resulted in the identification of new species, facilitated conservation efforts, assisted in food poisoning cases and contributed to the uncovering of large-scale seafood mislabeling.

KAPA Express Extract offers a fast, convenient and efficient alternative for the routine extraction of DNA from fish fins and flesh. The novel thermostable protease and buffer system has been designed for optimal tissue lysis and preservation of DNA extracts. The KAPA Express Extract enzyme and buffer are combined in a single tube with a 1 – 2 mm<sup>2</sup> punch from a fish fin, or a ~2 mm<sup>3</sup> piece of fish flesh. Tissue lysis is performed in a standard thermocycler, after which the reaction product is centrifuged and the DNA-containing supernatant recovered. This protocol eliminates excessive handling, thereby generating PCR-ready DNA in as little as 15 min. The process is easily scaled to handle multiple samples in a 96-well format.

KAPA2G Robust HotStart ReadyMix is ideally suited for the routine and reliable amplification of DNA extracted from fish tissues. This ready-to-use cocktail contains KAPA2G Robust HotStart DNA polymerase, a novel enzyme engineered for improved processivity and tolerance to common PCR inhibitors through a process of molecular evolution. Its improved tolerance to carry-over inhibition offers more reliable routine amplification of fish amplicons for direct sequencing. When used in combination with the KAPA Express Extract system, the turnaround times for DNA barcoding applications can be significantly reduced.



**Figure 1. Amplification and sequencing of the COI gene from different fish species using KAPA Express Extract and KAPA 2G Robust HotStart ReadyMix.**

A punch (2 mm<sup>2</sup>) was taken from the fin or tail of 15 different fish. In addition, a ~2 mm<sup>3</sup> piece of flesh was excised from each specimen. DNA extracts were prepared in a 96-well plate using KAPA Express Extract, as outlined on the next page. Of each extract, 2 µl was used directly (without quantification) in a PCR containing KAPA2G Robust HotStart ReadyMix and primers for the ~650 bp COI amplicon (Ivanova *et al.*, 2007). Reaction products generated from fin/tail punches (top panel) and flesh (bottom panel) are shown in gel image. PCR products corresponding to lanes 3, 5, 7, 9 and 12 (boxed) were subjected directly to standard Sanger sequencing using out-nested M13 primers (1 µl PCR product per 10 µl sequencing reaction). Sequence data was of a high quality and enabled the identification of each species, as shown in Table 1 on the next page. A portion of the sequence trace for sample 3 is given below the gel image.

## Results

To demonstrate the suitability of KAPA Express Extract and KAPA2G Robust HotStart ReadyMix for DNA barcoding of fish, DNA was extracted from a variety of fish samples. The ~650 bp COI fragment (Ivanova *et al.*, 2007) was subsequently amplified with KAPA2G Robust HotStart ReadyMix. Selected PCR products were subjected to standard Sanger sequencing. Fish species were successfully identified by BLAST analysis of sequence data. Typical PCR and sequencing results are given in **Figure 1**, and results from BLAST sequence similarity searches in **Table 1** on the next page.

## DNA barcoding from fish tissues

**Table 1:** Identity of fish samples investigated in this study.

Sample	Species (local name)	Similarity*
3	<i>Seriola lalandi</i> (Yellowtail amberjack)	100%
5	<i>Merluccius paradoxus</i> (Cape hake)	100%
7	<i>Sarda sarda</i> (Atlantic bonito)	99.6%
9	<i>Argyrosomus inodorus</i> (Silver Cob)	99.8%
12	<i>Seriola lalandi</i> (Yellowtail amberjack)	99.0%

\* Of sequence obtained for this sample to COI sequence by BLAST analysis.

## DNA extraction protocol

An overview of the KAPA Express Extract protocol using 96-well plates is given in **Table 2**. Punches (2 mm<sup>2</sup>) from the fin or tail or pieces of flesh (~2 mm<sup>3</sup>) were transferred directly to individual wells of a 96-well plate. A bulk KAPA Express Extract lysis solution was prepared as outlined in **Table 3**. Of this, 100 µl was added to each fish tissue sample. Several no template controls were included in the plate to control for contamination. The 96-well plate sealed with sealing film, placed in a thermocycler and the lysis protocol performed as indicated in **Table 2**. After the 15 min lysis protocol, the 96-well plate was centrifuged briefly to pellet debris. Of each lysate supernatant, 50 µl was transferred to a new 96-well plate. DNA extracts prepared in this way do not have to be quantified and may be used directly in a PCR. One extraction typically yields sufficient template for ~50 x 25 µl PCRs. DNA extracts may be diluted 1:5 in TE Buffer for long-term storage at -20°C. For small numbers of samples, individual lysis reactions may be performed in thin-walled PCR tubes.

**Table 2:** KAPA Express Extract protocol for fish tissues.

Step	Description
<b>Reaction setup</b>	<ol style="list-style-type: none"> <li>1. Transfer fish tissue samples directly into individual wells of a 96-well PCR plate.</li> <li>2. Prepare a bulk lysis solution by combining KAPA Express Extract Buffer, enzyme and PCR grade water, as outlined in <b>Table 3</b>.</li> <li>3. Add 100 µl bulk lysis solution to each sample.</li> </ol>
<b>Lysis</b>	<ol style="list-style-type: none"> <li>1. Seal plate and place in thermocycler.</li> <li>2. Incubate at 60 °C for 10 min.</li> </ol> (During this step, cells are lysed, nucleases and proteins degraded and DNA released.)
<b>Heat-inactivation</b>	Incubate plate at 95 °C for 5 min to inactivate the thermostable KAPA Express Extract enzyme.
<b>Sample recovery</b>	<ol style="list-style-type: none"> <li>1. Centrifuge plate for 1 min to pellet debris.</li> <li>2. Recover DNA-containing supernatant.</li> </ol>

## References

Ivanova, N. V., Zemlak, T. S., Hanner, R. H. and Hebert, P. D. N. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes* 7, 544–548.

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**Table 3:** Preparation of bulk KAPA Express Extract lysis solution.

Reaction component	Final conc.	Per 100 µl reaction	Per 96-well plate
PCR grade water	–	88.0 µl	8.80 ml
10X KAPA Express Extract Buffer	1X	10.0 µl	1.00 ml
KAPA Express Extract Enzyme (1 U/µl)	20 mU/µl	2.00 µl	0.20 ml

## PCR reaction conditions and cycling parameters

The KAPA2G Robust HotStart ReadyMix reaction setup and cycling parameters for the amplification of the ~650 bp COI fragment from fish tissues given in **Tables 4 and 5** were derived from the protocol of Ivanova *et al.* (2007), but adapted to the specific characteristics of the novel KAPA2G Robust enzyme. Overall cycling times were reduced considerably compared to the original protocol. With the combination of KAPA Express Extract and KAPA2G Robust HotStart ReadyMix, reliable DNA amplification from fish tissues can be achieved in ≤2 hours, compared to ≥1 day with conventional methods.

**Table 4:** KAPA2G Robust HotStart ReadyMix reaction setup for amplification of the COI gene from fish species.

Reaction component	Final conc.	Per 25 µl reaction <sup>1</sup>
PCR grade water	–	Up to 25.0 µl
2X KAPA2G Robust HotStart ReadyMix	1X	12.5 µl
Primer premix (10 µM)	0.15 µM (of each of 4 primers)	0.375 µl
KAPA Express Extract fish DNA extract	–	2.00 µl

<sup>1</sup> For smaller reaction volumes, scale down all volumes proportionally. Do not perform reactions >25 µl.

<sup>2</sup> A premix of the four degenerate COI primers (each with an out-nested M13 sequence) described by Ivanova *et al.* (2007) were made to facilitate reaction setup. The final concentration of each primer was increased to 0.2 µM to improve efficiency, whilst reducing the requirement for long annealing and extension times.

**Table 5:** KAPA2G Robust HotStart ReadyMix cycling parameters for amplification of the COI gene from fish species<sup>1</sup>.

Cycling step	Temperature & time	
Initial denaturation	3 min at 95 °C	
Denaturation	15 sec at 95 °C	x 40 cycles
Annealing	30 sec at 52 °C	
Extension	20 sec at 72 °C	
Final extension	10 min	

<sup>1</sup> These cycling parameters were specifically optimized for the fish COI DNA barcoding assay. For other primer-template combinations, please refer to the KAPA2G Robust HotStart ReadyMix Technical Data Sheet for general cycling parameters.